Diesel Emission Induced Lung Toxicity Responses

NO_2/NOx ratio versus Particulate Matter

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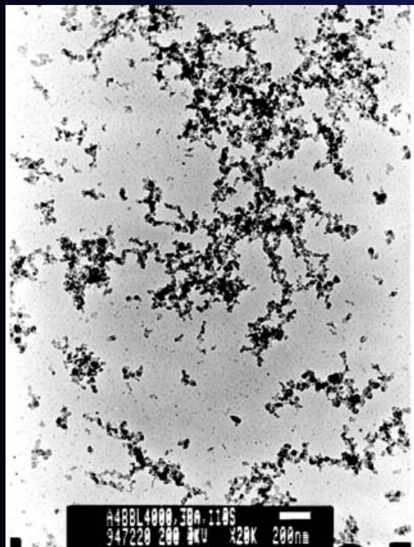
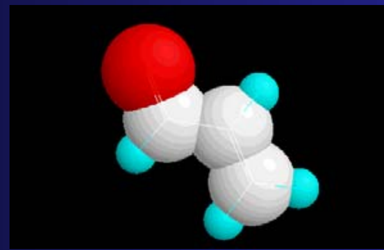
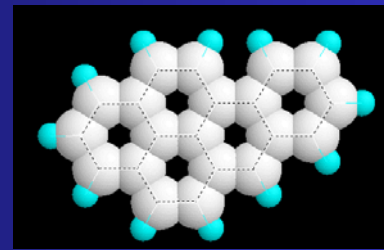
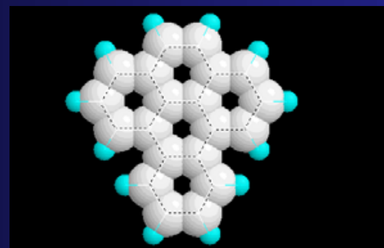
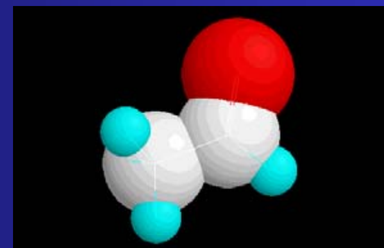
Impact of Complex Aerosols on Lung Tissue

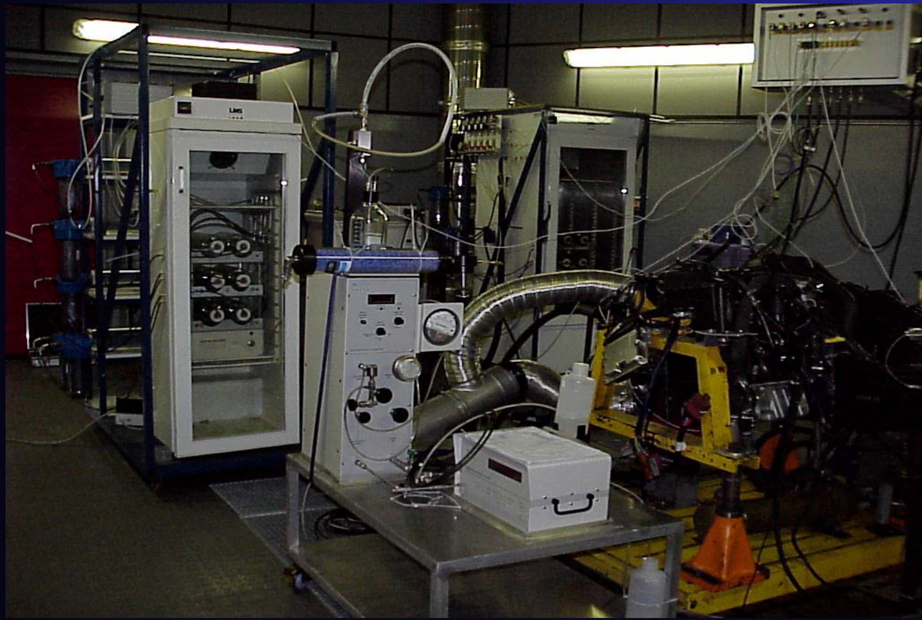


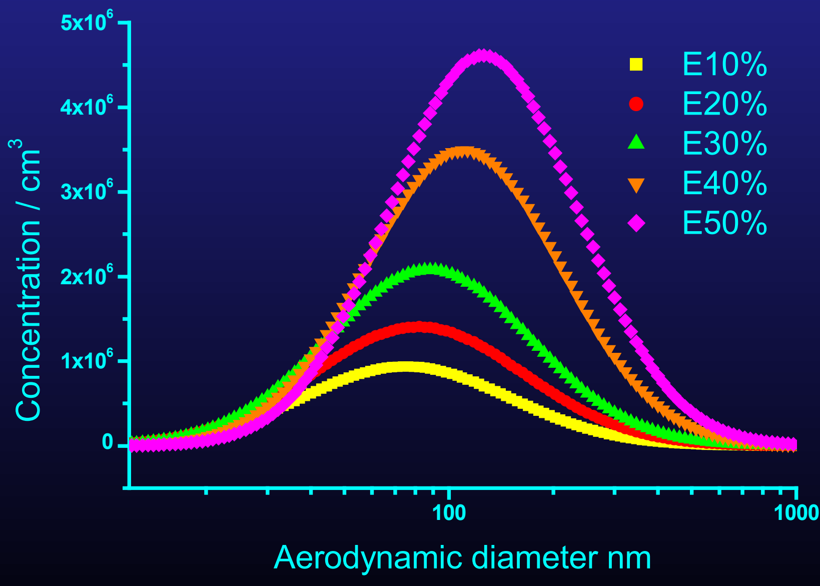












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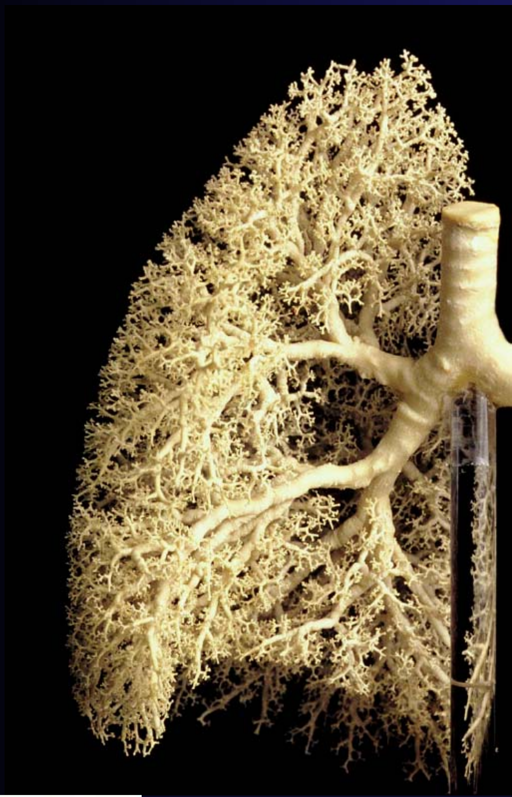
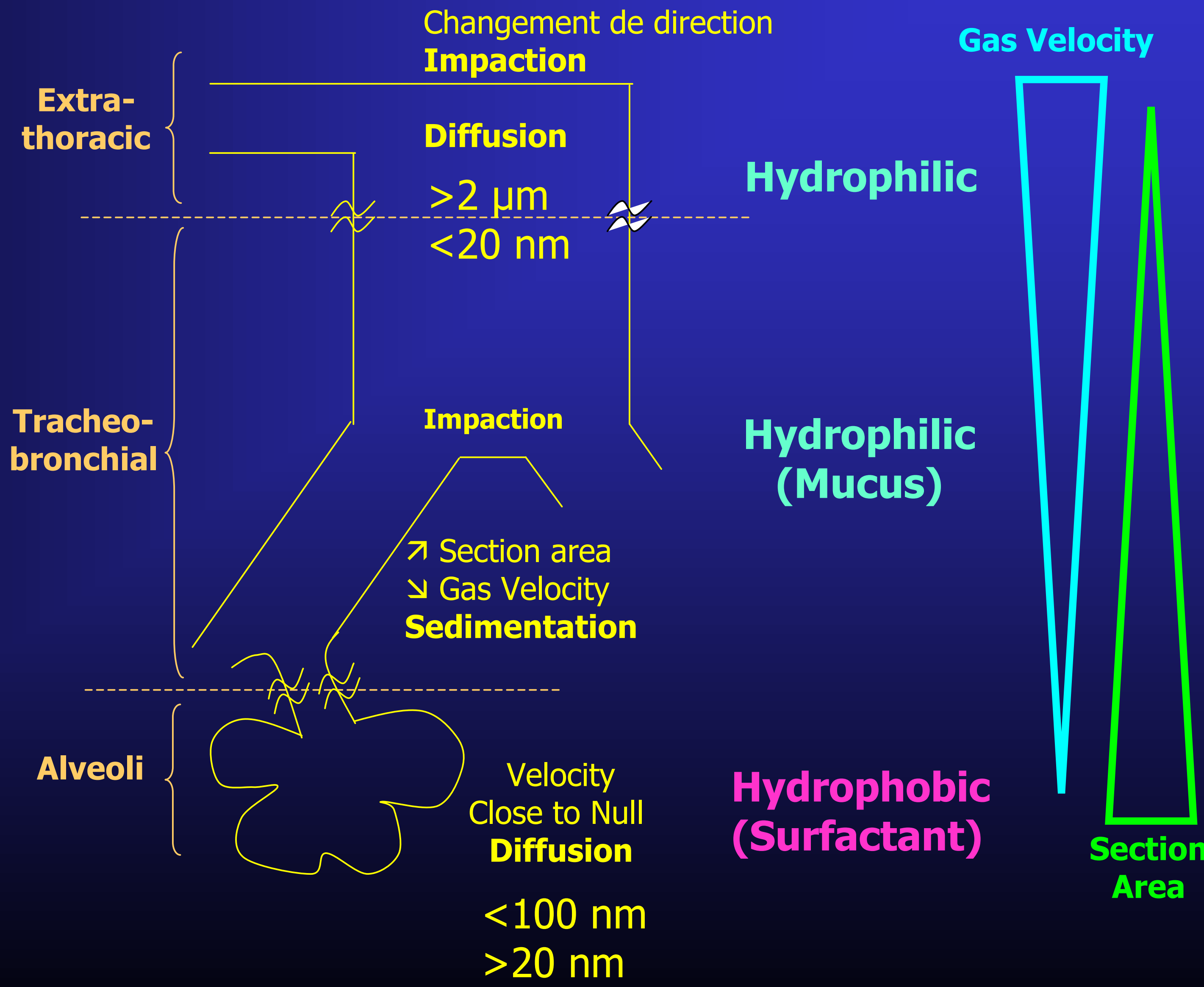
Respiratory tract Gas Velocity and PM deposition

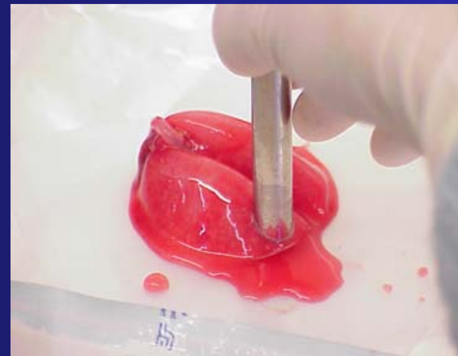




Rat Lung Slices Preparation And Dynamic Air/Liquid Culture

Agarose filled lungs

Peripheral lung coring



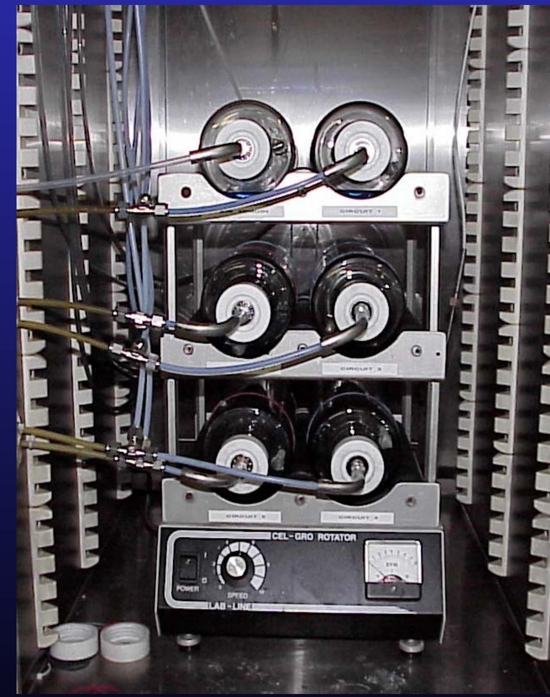
Brendel/Vitron Slicer

Diam 8mm - Thick 1500µm

Opened cap vials

Slices on Cylindrical Inserts

Flow-through Rotating Exposure Chambers



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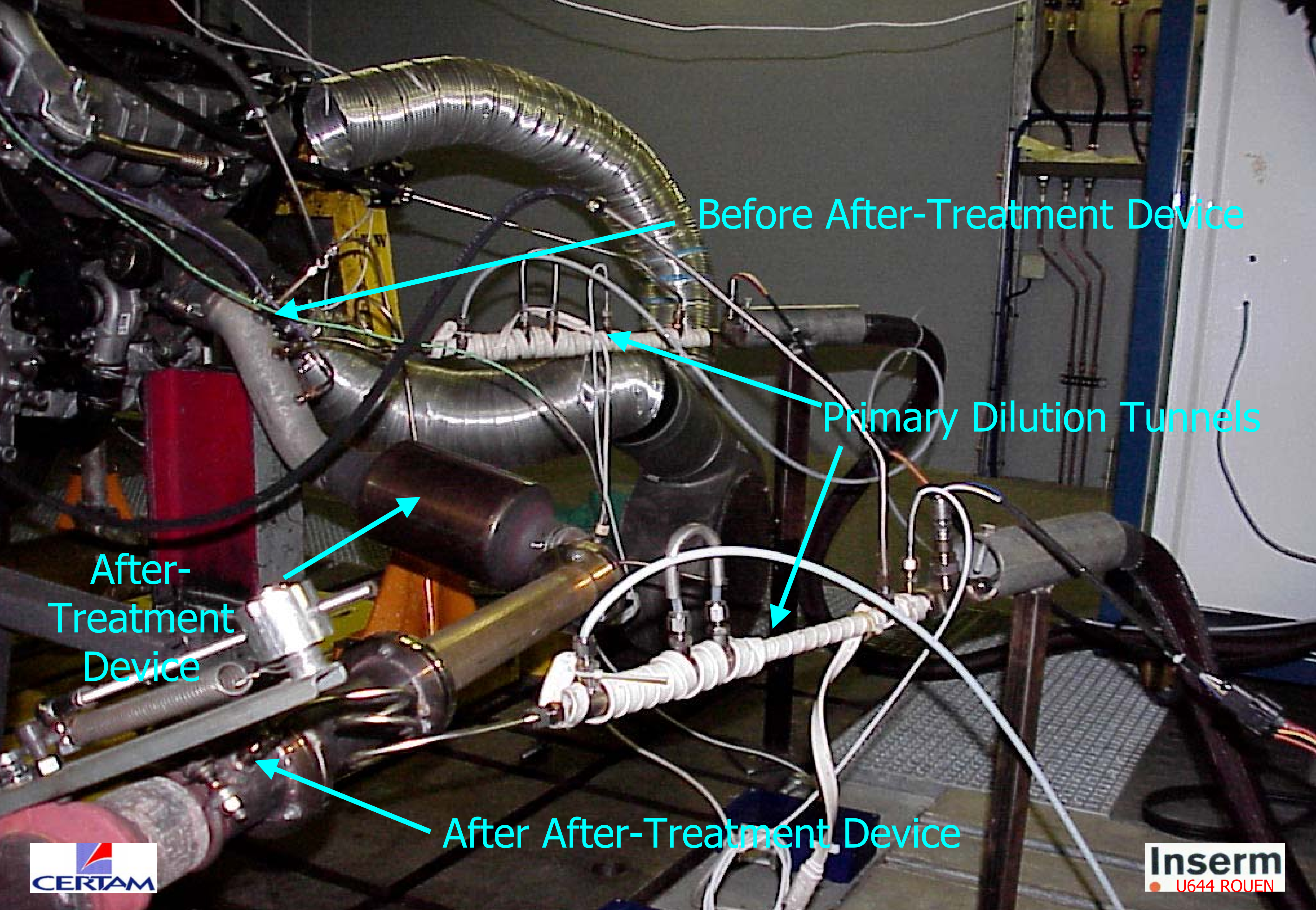
OPTIMISED
Combustion Aerosol Sampling
and
Dilution Systems


Before After-Treatment Device
Primary Dilution Tunnels
After-Treatment Device
After After-Treatment Device
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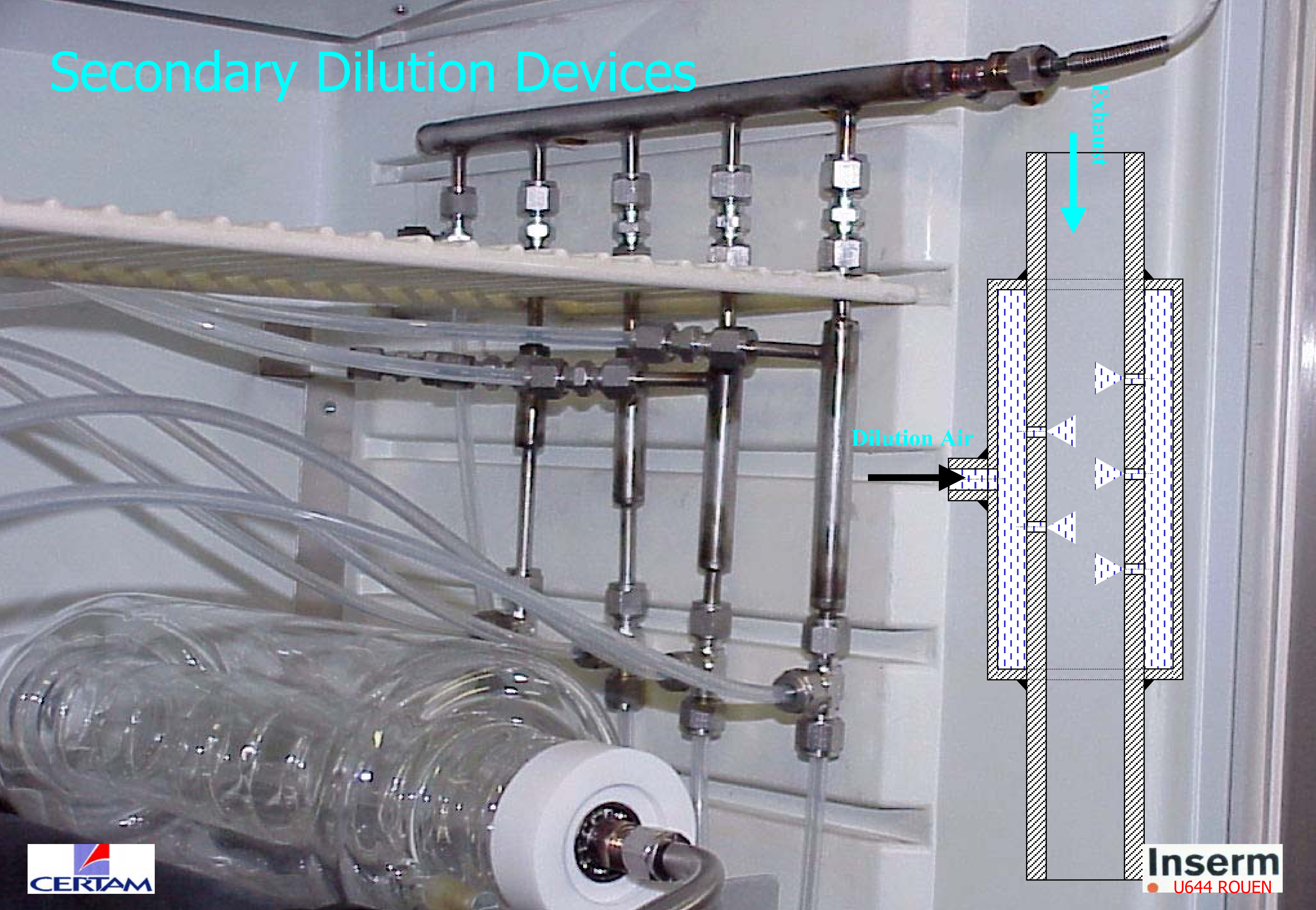
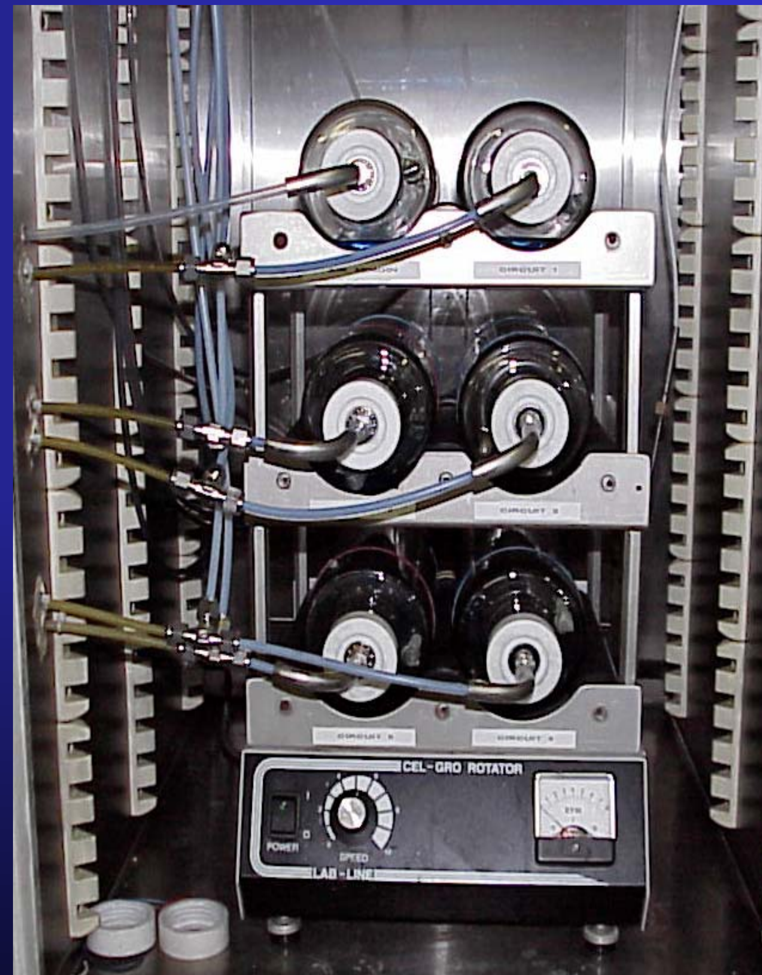
Secondary Dilution Devices



Standardized Dilution and Exposure Systems



Organotypic Cultures

Advantages of Sampling and Exposure Systems

* No alteration of both gazeous phase and PM physicochemical properties

* Interactions Aerosol/Biological sample mimicking the in vivo situation (sedimentation and diffusion)

* No Alteration of pollutant Bioavailibility

* Global Approach of Exhaust impact

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Toxicity Endpoints

-**Cell viability** :
Intracellular ATP content

- **Oxidative stress and Detoxication** :
Intracellular glutathione content (GSH)
Enzyme activity of SOD, Catalase, GPx, GST
8-hydroxy-2′-deoxyguanosin (histological staining)

- **Inflammatory response** :
TNFα (release in culture medium)
ICAM-1 (histological staining)

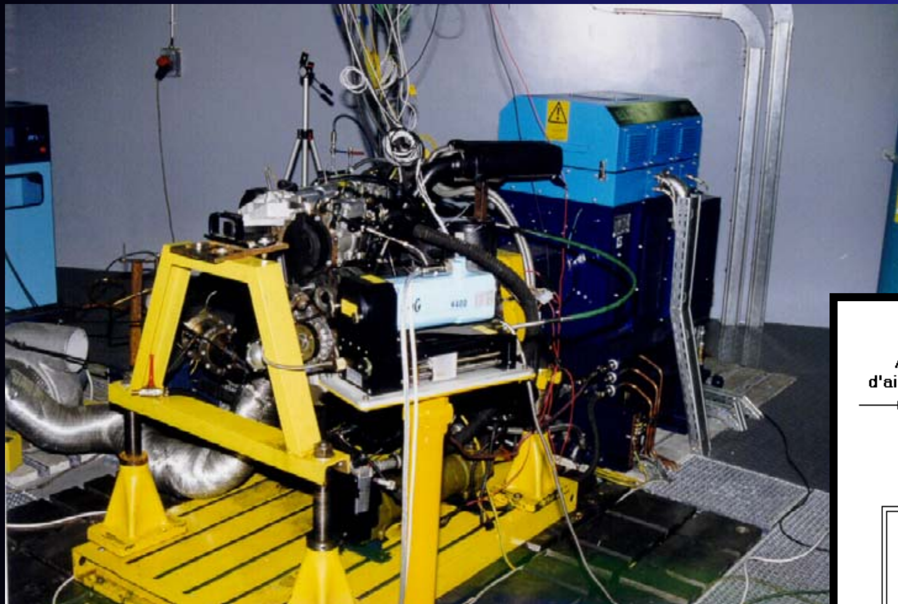
-**Apoptosis :**
-Nucleosome Assay
-TUNEL (histological staining)
-DNA Ladders

Comparative Impacts on Lung Slices

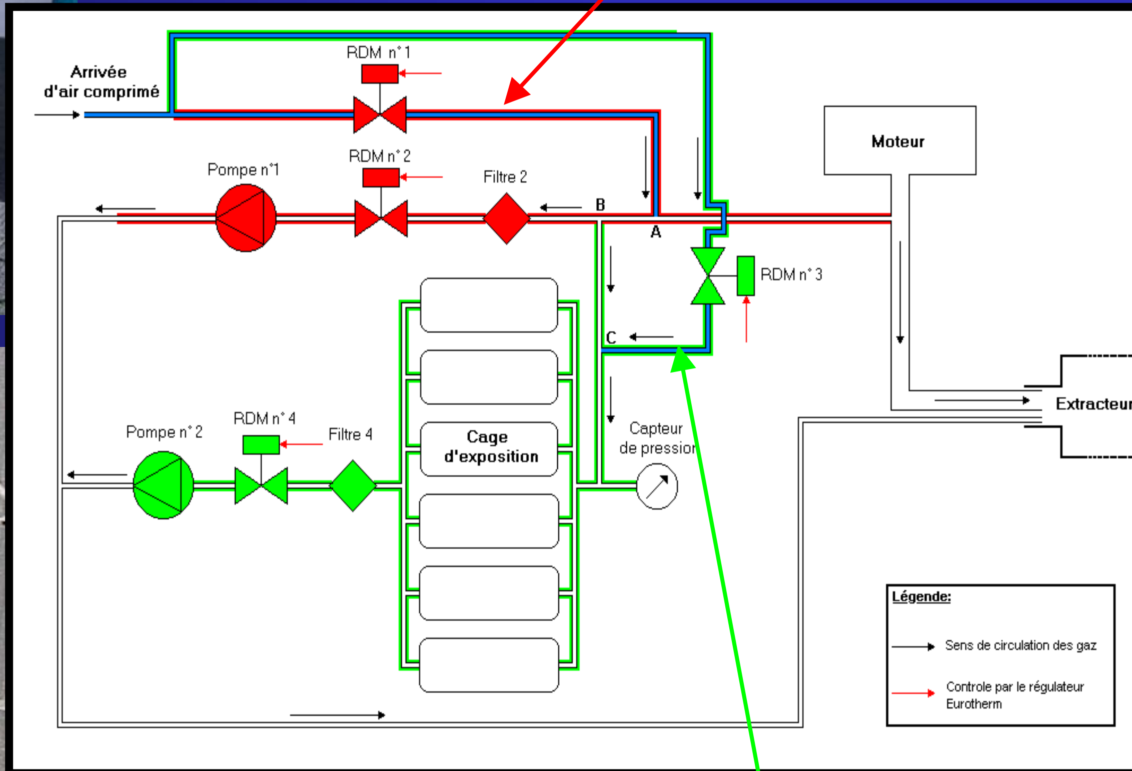
	Diesel Exhaust (Low NO_2/NO ratio)		Suspended DEP / Medium	Suspended DEP / Tween	
	Total	Filtered	Total	Total	
Filtrate					
ATP	=	=	=	=	=
GPx	+	+	=	=	=
GST	+	+	=	++	++
Catalase	+	+	=	-	-/=
GSH	---	--	=	=	=
SOD	-	-	=	++	++
TNFα	++	=	=	=+	++
Nucleosomes	++	=	=	=	=
TUNEL	+++	=	=	=	=
DNA Ladders	++	=	=	=	=
8-OxoG	+++	++	=	=	=
		Gas Phase	No Effect		Desorbed

in vivo

Sampling and dilution of Engine Exhausts



1rst dilution loop



2nd dilution loop

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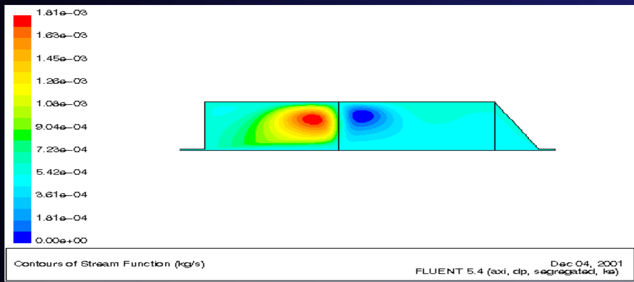
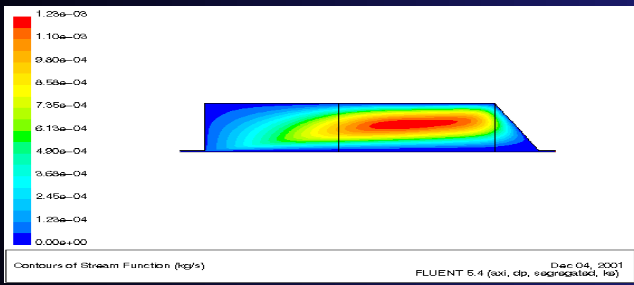
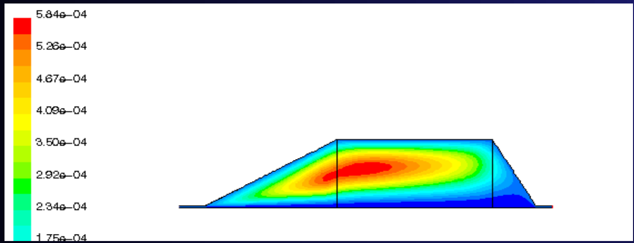
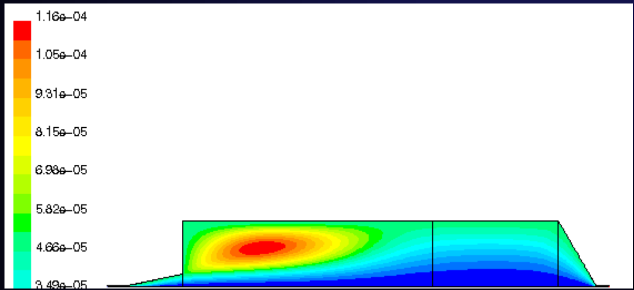
Design of Inhalation Cages for Vigile Unconstraint Rodents

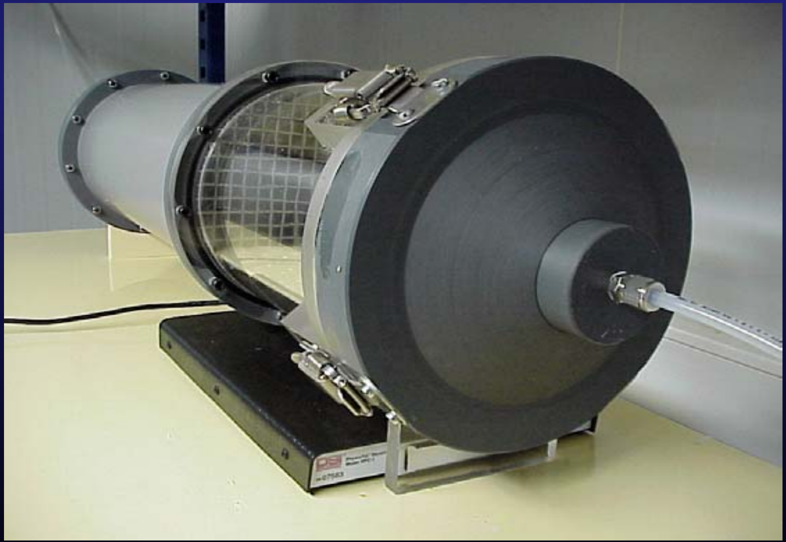
Modelization with FLUENT 2D











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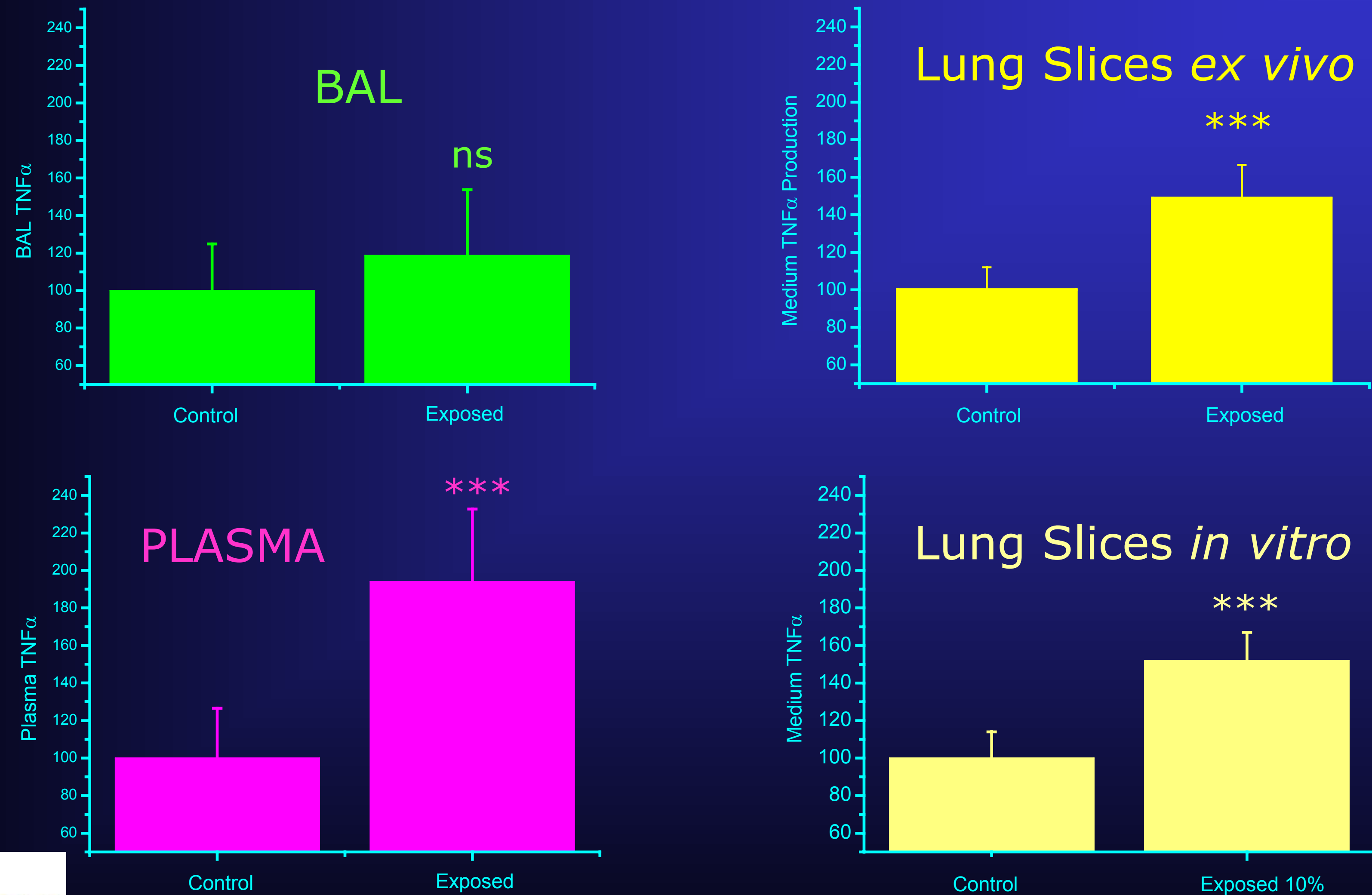
Comparison of in vivo and in vitro lung responses

Could Rat lung slices in bi-phasic air/liquid culture be used as a surrogate for in vivo aerosol inhalation toxicology ?

Inflammation : TNFα Production



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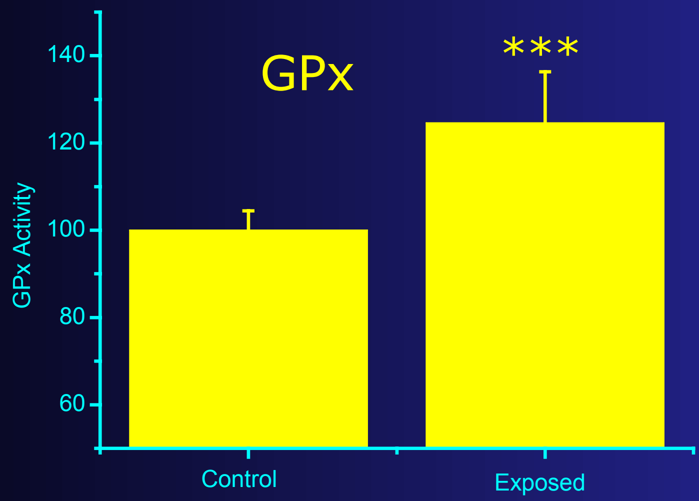
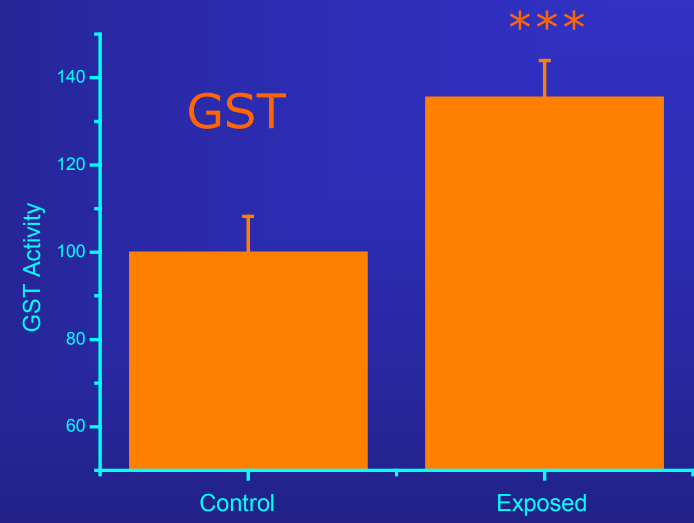
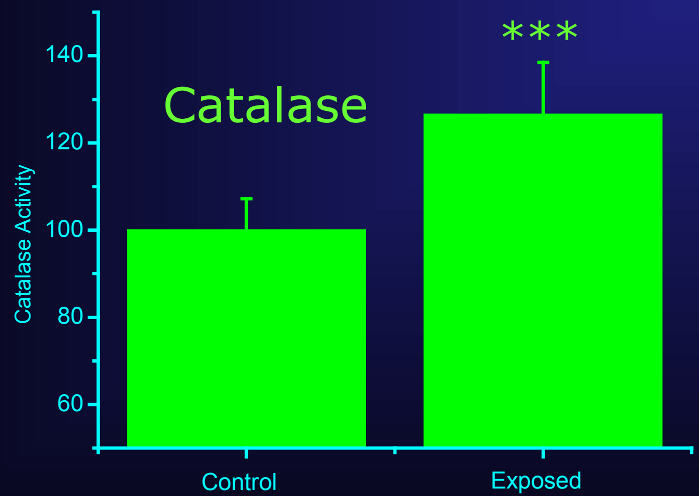
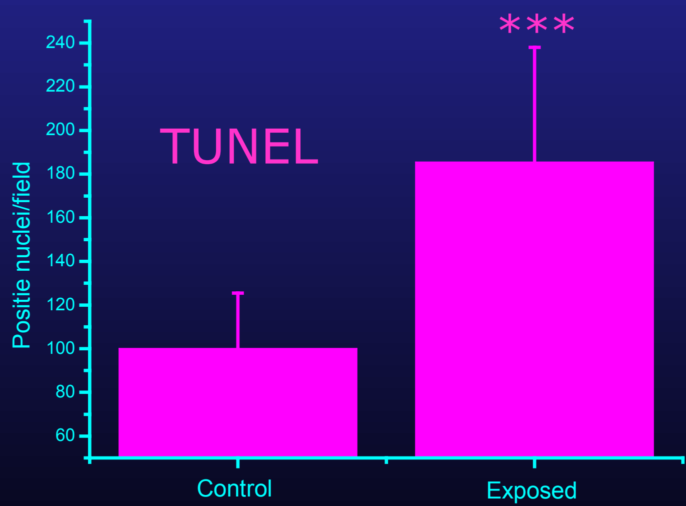
Lung Tissue Investigations









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Lung Tissue Investigations

Excellent correlations between *in vitro* and *in vivo*
Lung Toxicity patterns
with continuous exposure to diluted exhausts

DNA alteration, Inflammation and Oxidant stress

Use of Rat Lung Slices in bi-phasic air/liquid culture
as a surrogate for in vivo aerosol inhalation toxicology Yes

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Modulation of Engine Emissions Quality

Fuel, Engine load, Filter, « Treatment »

Modulation of CO, NOx, HC, PM

Diesel Engine Emissions

⇨ Exhaust Characteristics

Gas Phase	A	B	C	D	E	F	G	H
HC (ppm)	29	29	19	19	10	10	0	0
CO (ppm)	137	137	0	0	0	0	0	0
NOx (ppm)	423	423	406	406	467	467	484	484
NO_2 (ppm)	**24**	**24**	**106**	**106**	**191**	**191**	**260**	**260**
NO (ppm)	399	399	300	300	277	277	224	224
NO_2/NO	**0,06**	**0,06**	**0,35**	**0,35**	**0,69**	**0,69**	**1,16**	**1,16**

Oxidant Potential →

Smoke Index	A	B	C	D	E	F	G	H
FSN	1.8	ND	1.8	ND	0.7	ND	0.7	ND
mg/m^3	44	ND	44	ND	12	ND	12	ND

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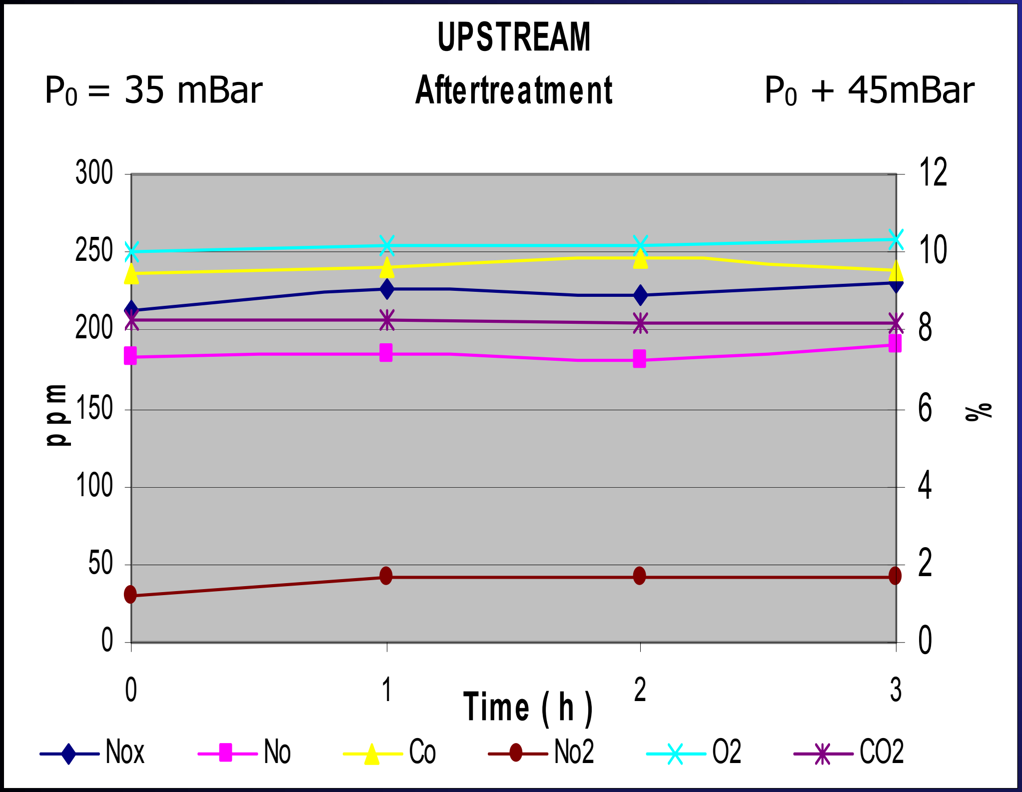
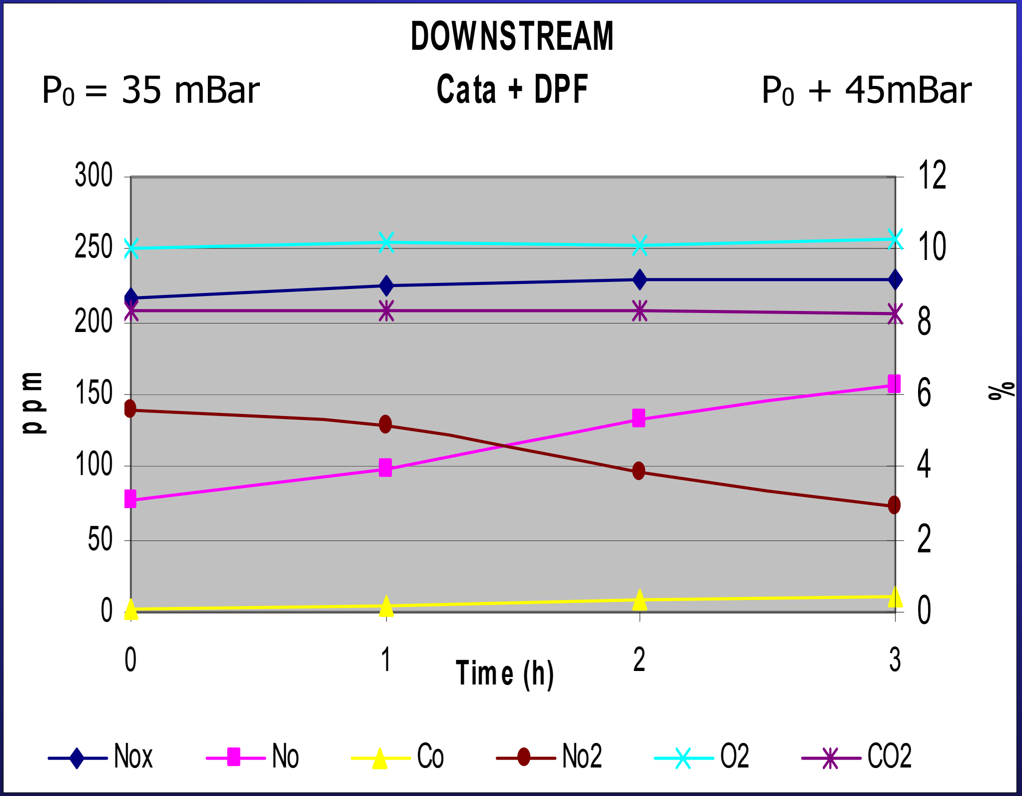
Pollutant Emission Time Evolution





No Change in Total NOx But Changes in NO_2 and NO proportions

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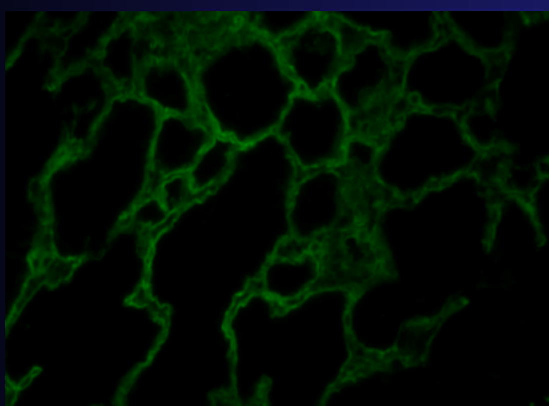
INFLAMMATORY RESPONSE

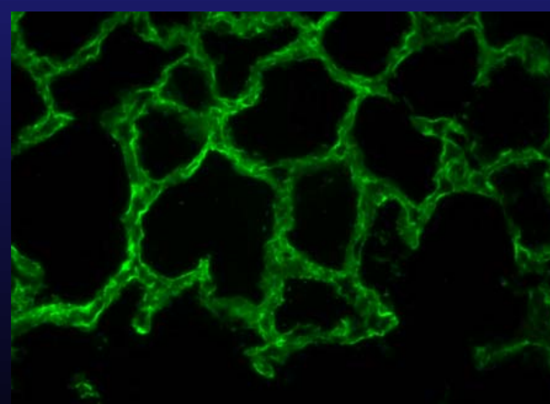
Diesel Emission

	TNFalpha Production	ICAM-1 Expression		
A	↗	↗	⇨ Almost Exclusively PM Phase	Low NO_2/NO
B	=	↗		
C,D	↗	↗	⇨ Gas Phase	
E,F	↘	↗	⇨ Gas phase	High NO_2/NO
G,H	↘	↗		

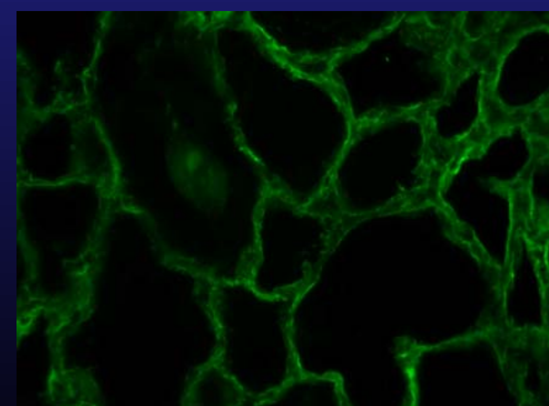
ICAM-1



Control



Emissions A, C, D, E, F, G, H

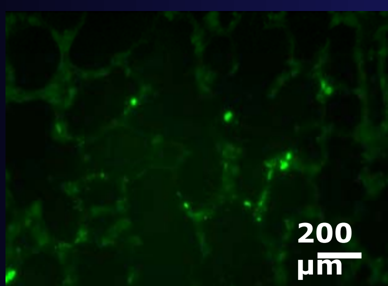


Emission B

DIESEL Emissions : APOPTOSIS

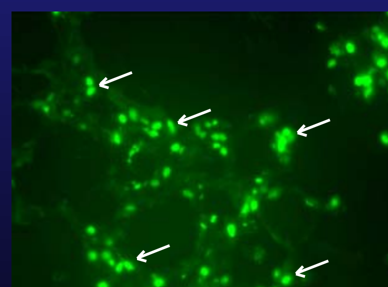
Effluents Diesel

	Nucleosomes	DNA Scales	TUNEL		
A	↗	+	↗	⇨ **PM effect**	Low NO_2/NO
B	=	-	=		
C,D	↗	+	↗	⇨ **Gas Phase Effect**	
E,F	=	-	=	⇨ **No apparent Effect**	
G,H	=	-	=	⇨ **Necrosis ?**	High NO_2/NO

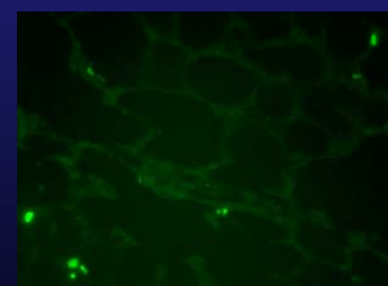


Control

Tunel Labeling



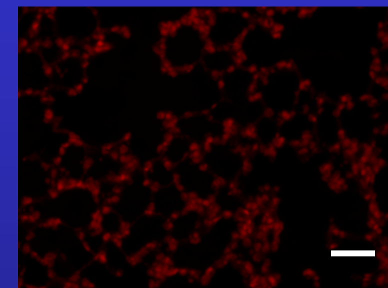
A, C and D



B, E, F, G and H

DIESEL Emissions and DETOXICATION Systems

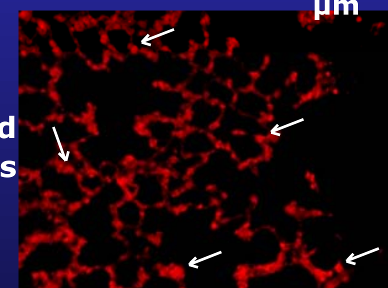
		A,B	C,D	E,F	G,H
Detoxication Systems	GSH	↘	↘	↘	↘
	GST	↗	↗	=	=
Oxidant Stress	GPx	↗	↗	↗	↗
	Catalase	=	=	↗	↗
	MnSOD	↗	↗	=	=
	8-oxoguanine	+	+	+	+
		Low NO_2/NO		High NO_2/NO	



Control

200 µm

Oxidized Guanines



Diesel

- ⇨ **Two Distinct Profiles According to NO_2/NO Ratio**
- ⇨ **Predominant impact of gaseous phase**

CONCLUSION AND SUGGESTIONS

Untreated Raw Diesel Engine Emissions
exhibit low oxidant capacity
and exert their Toxicity Mainly through PM matter

The Apparent Toxic Impact of PM is « Modulated »
By the Oxidant Potential of the Gas phase

NO_2/NO ratio has been chosen as a marker
of exhaust global oxidant potential

Inflammation and Oxidant Stress, but no Cytotoxicity,
Induced by PM at low <u>NO_2/NO ratio (<0.1)</u>

Oxidant Stress, Cytotoxicity and
No Inflammation
Due to the Gaseous Phase
No more apparent impact of PM
For <u>NO_2/NO ratio > 0.2</u>

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Several Strategies for After Treating Diesel Engine Emissions rely on increasing the Oxidant potential of the Gas phase. Health Impact Concerns may Arise from these Strategies

Beside Total Nox measurements,
NO_2 and NO proportions should be Carefully monitored as a potential pertinent marker of Diesel Engine Emission « Health Safety »

This may help to no regret « health based » industrial strategies and air quality regulations

Acknowledgements to Coworkers

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